

DISCOVERY OF NATURAL HYBRIDIZATION IN TENEBRIONID BEETLES (COLEOPTERA: TENEBRIONIDAE)¹

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ABSTRACT: Hybrids between *Blaps gibba* (distributed in Southern Europe) and *B. mucronata* (widely distributed in Europe and imported to North America) from some small Italian islands are described. This is the first ascertained case of natural hybridization in tenebrionid beetles. Taxonomic and phylogenetic implications are discussed.

Current knowledge of hybridization in Coleoptera is very fragmentary and generally restricted to the occurrence of hybrid individuals arising from captive breeding of parent species (Sokoloff 1977, Thiele 1977, Crowson 1981, Brownlee & Sokoloff 1988, Suzuki et al. 1988a, 1988b; Nilsson & Johnson 1993, Auvray & Auvray 1998). Most of the research on natural hybrids among Coleoptera deals with the presence of hybrid zones between parapatric species (Kubota 1988, 1991, 1996, Mossakowski et al., 1986, 1990), whereas little is known about the occurrence of hybrid specimens between sympatric species (e. g., Casale et al. 1982, Pilon 1994). The natural hybridization between sympatric beetles is very difficult to study, because a correct evaluation of hypothesized hybrid specimens requires a bulk of knowledge that is rarely available. In particular, detailed information on the general distribution, seasonal occurrence, ecological preferences, and activity rhythms of the suspect parent species is required.

In fact, morphologically intermediate specimens have been described from various beetle families, but a conclusive evidence that they are natural hybrids has been rarely provided because of the lack of detailed information on the bionomics and intraspecific morphological variations of their parent species (Casale et al. 1982, Carpaneto & Piattella 1988, Pilon 1994).

As to the family Tenebrionidae, hybridization is known to occur between *Tribolium* species in rearing conditions (e. g. Suzuki et al. 1988a, 1988b). However, as far as we know, no natural hybrids have been described in this family. In this paper, we report the first instance of natural hybridization between two tenebrionid species belonging to the genus *Blaps*.

MATERIALS AND METHODS

We examined four male specimens showing intermediate characters between *Blaps gibba* and *B. mucronata*. Two specimens were collected on the

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Island of Alicudi (Sicily, Italy, 29 - VIII / 5 - IX - 1994, U. Pessolano leg.; S. Fattorini's and P. Leo's personal collections); the third specimen on the Island of Santo Stefano (Latium, Italy, 5 - IV - 1966, V. Cottarelli leg.; Museum of Zoology, Rome University "La Sapienza") and the fourth specimen on the Island of Ventotene (Latium, Italy, VII - 1988, M. Pischedda leg.; Museum of Zoology, Rome University "La Sapienza"). To test if they could be hybrids between *Blaps gibba* and *B. mucronata*, we carried out a morphometric analysis on specimens of both species. A total of 16 male specimens of *B. gibba* and 16 male specimens of *B. mucronata*, collected from sites representative of all their ranges, was examined. Only male specimens were chosen in order to more easily compare specimens using the same set of characters. Measurements were made using an ocular grid and recorded in millimeters. The analysis was based on a set of five ratio characters listed in Table 1. The use of ratios has been criticized by some authors (Atchley et al. 1976), but supported by others who pointed out the value of using ratios to measure shape particularly when the raw data are logarithmically transformed (Hills 1978, Dodson 1978, Belfiore 1996). In the present study all numerical computations were performed on log-10 transformed ratios. Differences in morphometric ratio characters between *B. gibba* and *B. mucronata* were tested by using both the univariate *t*-test for each character considered individually, and the multivariate Hotelling T^2 test for all characters considered together. In addition, qualitative male characters, like the presence of thick bristles between the first and second abdominal sternites, the sinuosity on the fore tibiae, and the general feature of genitalia were studied.

A discriminant analysis was also applied to compare hybrid specimens, *B. gibba* specimens and *B. mucronata* specimens. For this analysis, the following log-10 transformed morphometric characters were initially taken in account as possible predictors: PRL, PRW, TIL, TIW, ELL, ELW, 3ANTL, 3ANTW, 4ANTL, 4ANTW (acronyms as in Table 1). To drastically prune this large number of variables, a single factor ANOVA was carried out and the *F* statistics were used to rank the potential predictors. At this step, ELL was excluded,

Table 1. Ratio characters used in morphological analyses.

Ratios	Definitions
PRL/PRW	Medial pronotal length / maximum pronotal width
TIL/TIW	Maximum length of fore tibiae, excluding teeth / width of fore tibiae at 1/2 of its length
ELL/ELW	Elytral length from pronotal base to the elytral apex / maximum elytral width
3ANTL/3ANTW	Medial length of 3rd antennal article / width of 3rd antennal article at 1/2 of its length
4ANTL/4ANTW	Medial length of 4th antennal article / width of 4th antennal article at 1/2 of its length

because it was not significant. As a second step, the remaining variables were tested for correlation by using the Pearson product moment coefficient to identify redundancies. If two variables were highly correlated ($r \leq -0.75$ or $r \geq 0.75$), we retained the one with the largest F statistic. In this way, the number of predictors was reduced to PRW and 3ANTL. Specimens were assigned to three groups (hybrids: 4 specimens; *B. mucronata*: 16 specimens; and *B. gibba*: 16 specimens), and PRW and 3ANTL were used as variables.

In all tests, a minimum probability level of $P < 0.05$ was accepted. Statistical analyses were performed using STATISTICA software (version 4.5, 1993).

RESULTS

B. mucronata and *B. gibba* can be clearly distinguished by the different shape of the aedeagus, which is slender in *B. mucronata* (Fig. 1). Also, *B. gibba* shows two important sexually dimorphic characters, absent in *B. mucronata*. The male of *B. gibba* has a clear, large sinuosity on the internal surface of the fore tibiae (Fig. 1) (sometimes recognizable, even if very reduced, also in females) and a group of thick bristles between the first and second abdominal sternites. Male and female specimens of *B. mucronata* have neither the sinuosity on the fore tibiae nor the bristles between the first and second abdominal sternites.

Means, standard errors and standard deviations of each of the five morphometric characters (ratios) measured in the 16 male specimens of both species and the four male putative hybrid specimens are compared in Fig. 2. As revealed by a multivariate Hotelling test, the two parent species significantly differ for these characters ($T^2 = 395.556$, $F(5, 26) = 68.563$, $P < 0.00000$). Using a univariate (t -test) analysis for these characters, significant differences between *B. gibba* and *B. mucronata* were observed for all characters but ELL/ELW (Table 2).

For all characters the putative hybrid specimens show values intermediate between those of *B. gibba* and *B. mucronata* (Fig. 2). Also, the putative hybrid specimens show intermediate features between the two parent species for the general shape of

Table 2. Mean values and standard deviations of morphometrics variables in males of *B. gibba* ($n = 16$) and *B. mucronata* ($n = 16$) (Data are presented as log-10 transformed ratios). Acronyms as in Table 1.

	<i>B. gibba</i>		<i>B. mucronata</i>		t -value	df	P
	Mean	SD	Mean	SD			
PRL/PRW	-0.165	0.020	-0.126	0.012	-6.816	30.000	0.000
ELL/ELW	0.208	0.022	0.218	0.007	-1.790	30.000	0.084
TIL/TIW	0.913	0.033	1.018	0.021	-10.735	30.000	0.000
3ANTL/3ANTW	0.606	0.029	0.755	0.022	-16.546	30.000	0.000
4ANTL/4ANTW	0.173	0.042	0.362	0.022	-16.081	30.000	0.000

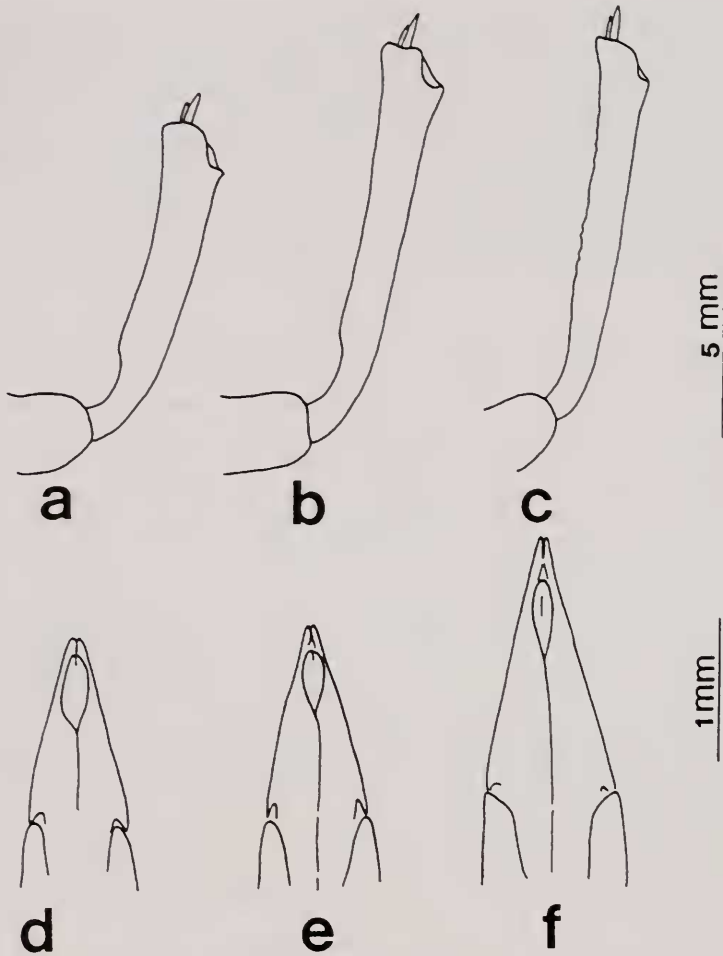


Fig. 1 - Male right fore tibia (A, B, C) and aedeagus (D, E, F) of *B. gibba* (A, D), a hybrid specimen from Alicudi (Italy) (B, E), and *B. mucronata* (C, F).

both the parameres and the fore tibiae (Fig. 1). In particular, the fore tibiae of putative hybrids have an intermediate length coupled with the sinuosity exclusive of *B. gibba*. However, in contrast to *B. gibba*, putative hybrids do not have thick bristles between the first and second abdominal sternites.

The use of discriminant function analysis as a classification technique gave a clear discrimination between the two species, while the putative hybrids occupy an intermediate position (Fig. 3). The first discriminant function had an eigenvalue of 16.685, accounting for 99.86% of variance. The second function had an eigenvalue of 0.023. The second function is not significant and does not separate groups (Fig. 3). Based on standardized coefficients, both 3ANTL

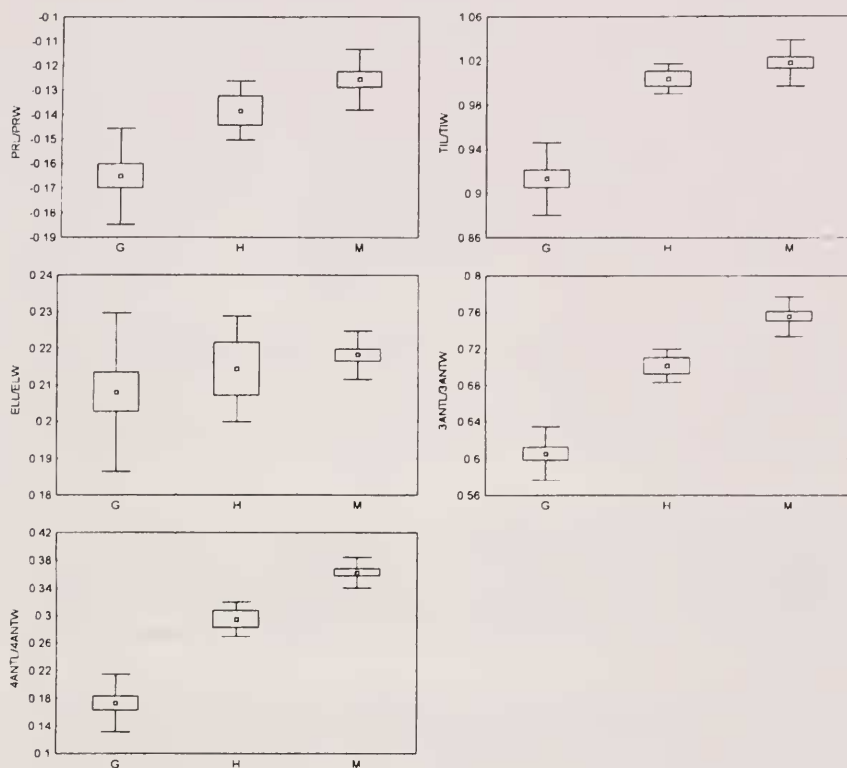


Fig. 2 – Mean values (middle points), standard errors (boxes) and standard deviations (whiskers) of five ratio characters of *B. gibba* (G, n = 16), hybrid specimens (H, n = 4) and *B. mucronata* (M, n = 16). Acronyms as in Table 1.

(1.439) and PRW (-1.349) were relevant for the first function, showing lower values (-0.416 and -0.651, respectively) for the second function. Very low Wilks' lambda value for the first derived function and the related value of chi-square indicated that a very high discriminating power exists in the two parameters being used (Fig. 3). The specimens have, therefore, significantly different morphometric character values. Also, 100 % of specimens of *B. gibba*, *B. mucronata* and putative hybrids are correctly classified.

DISCUSSION

B. gibba and *B. mucronata* are two morphologically well characterized species, from both a qualitative and a morphometric point of view. By qualitative and quantitative analysis, putative hybrids showed not only intermediate character states between the two parental species, but also an unique combination of qualitative characters of both species.

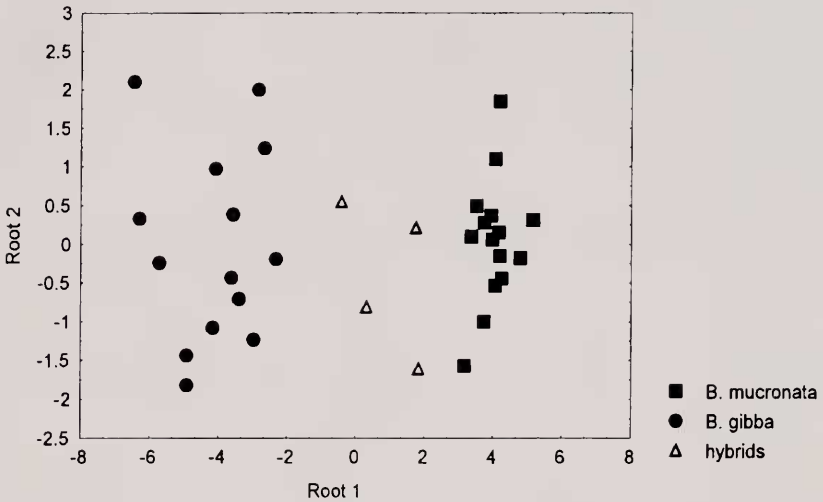


Fig. 3 – Scatterplot of canonical scores obtained from Discriminant Analysis based on two morphological characters (see methods for details). Discriminant Function Analysis Summary: Wilks' Lambda = 0.055, $F(4,64) = 52.061$, $P < 0.0000$, percentage of correct classifications: 100. Test of function 1 through 2: canonical $R = 0.971$, Wilks' lambda = 0.055, chi-square = 94.108, $df = 4$, $P < 0.000$. Test of function 2: canonical $R = 0.151$, Wilks' lambda = 0.977, chi-square = 0.745, $df = 1$, $P = 0.388$.

These results, coupled to the following distributional and ecological information, strongly support that these specimens are actually natural hybrids.

B. mucronata is a widely distributed species, occurring through Central Southern Europe and the Anatolian Peninsula (imported to North America), while *B. gibba* occurs in the Italian Peninsula, Sicily, Sardinia, Corsica, the Balearic Islands, and Dalmatia (Fattorini & Leo 2000). Both species are locally common and anthropophilic, being often encountered under stones at the base of demolished buildings, in archeological and urban areas, as well as in cellars and stables. In Italy, they show a clearly sympatric distribution. Also, they are sometimes syntopic, and specimens of both species can be easily found together, e. g. in a given cellar or even under the same stone in urban habitats (pers. obs. in Central Italy). A study performed on *B. gibba* and *B. mucronata* specimens living in the same hole in an old building in a rural area in Central Italy (near Spoleto, Perugia) revealed that they are attracted by the same food and share the same nocturnal activity (S. Fattorini, pers. obs.). As to the occurrence of *B. gibba* and *B. mucronata* on the islands where the hybrids have been discovered, the presence of both *B. gibba* and *B. mucronata* on Alicudi is reported by Marcuzzi (1970), as well as both species are reported for Santo Stefano and Ventotene by Canzoneri (1976).

All these facts concur to provide evidence that the parent species share the same ecological preferences, seasonal distribution, activity rhythms and, more important, that they actually occur on the islands where the hybrids have been found. Co-occurrence of both *B. gibba* and *B. mucronata* in the same habitats, sometimes taking shelter under the same stone, can play an important role in determining interspecific matings. The presence of hybrids also suggests that these two species have low pheromonal specificity. Females of various *Eleodes* species are known to equally attract males of different species (Allsopp 1980). Most probably, *Blaps* females are also able to attract males of different species.

Although the parental species are distributed commonly and sympatrically in broad areas, the hybrids have been rarely found from only the above cited small islands. In the last decade, we examined thousands of *B. gibba* and *B. mucronata* from most of their ranges, but no hybrid specimens were ascertained beside those discussed here. Therefore, we believe that small islands, due to their reduced areas and habitat diversity, probably favor hybridization as a result of an increase of syntopy.

The presence of hybrid species in tenebrionid beetles has both taxonomic and systematic important implications. As a whole, the hybrid specimens show a combination of characters exclusive of each parent species and intermediate characters. As these characters are used in identification keys (Allard 1880, Seidlitz 1896, Español 1961), hybrid species like these do not fit with keys and could be erroneously classified or regarded as unknown species. Also, based on their very different morphology, *B. gibba* and *B. mucronata* have been assigned to different, unrelated species groups or subgenera by various authors (e. g., Allard 1880, Seidlitz 1896, Gebien 1937). Thus, from a phylogenetic point of view, discovery of natural hybrids allows to establish unsuspected relationships among morphologically very different species.

ACKNOWLEDGMENTS

The authors express their appreciation to Prof. M. A. Bologna ("Roma Tre" University, Rome) and to Prof. A. Vigna Taglianti, "La Sapienza" University, Rome, for reviewing the manuscript. The beetle donations of Dr. U. Pessolano were much appreciated. This research was supported by a grant from the Italian Ministero dell'Università e della Ricerca Scientifica e Tecnologica (60% "Roma Tre" University). This is publication no. 48 of the project "Ricerche sulle popolazioni insulari promosse e finanziate dal Consiglio Nazionale delle Ricerche, Isole Ponziane".

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